ABSTRACT:

A gene encoding a-L-arabinofuranosidase (AnabfA) from Aspergillus niger ATCC 120120 was successfully cloned and expressed in Pichia pastoris under the control of the AOX1 promoter. The effect of cultural conditions on recombinant AnabfA production was studied and the enzyme was expressed as a soluble protein. Recombinant AnabfA was expressed as an active enzyme at 28°C when cultured in BMMY medium (pH 6.0) and induced with 2% methanol every 24 h. Maximum activity was observed 5 days after induction. The purified recombinant AnabfA before and after treatment with PNGase F migrated by SDS-PAGE had relative molecular masses of about 83 and 66 kDa, respectively, suggesting that the AnabfA contains N-linked oligosaccharides. Characterisation of the purified recombinant AnabfA showed an optimum temperature and pH of 50°C and 4, respectively. The enzyme was stable at a pH of 3 to 6 and retained more than 80% of its activity after pre-incubation at 40°C for 30 min. The recombinant AnabfA activity was stimulated by K +, Mn 2+, Na 2+ and triton X-100 and was strongly inhibited by Cu 2+ and Fe 2+ and the enzyme activity was relatively unaffected by Ca 2+, CO 2+, Mg 2+ and EDTA. The K m and V max of the purified recombinant AnabfA activity towards ?NPA were 0.93 mM and 17.86 µmol/ml/min, respectively.